

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Vadim Gladyshev Publications

Biochemistry, Department of

2008

A Functional Link between Housekeeping Selenoproteins and Phase II Enzymes

Aniruddha Sengupta

Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, CCR, NCI, NIH, Bethesda, MD

Bradley A. Carlson

Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, CCR, NCI, NIH, Bethesda, MD

James A. Weaver

Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, CCR, NCI, NIH, Bethesda, MD

Sergey V. Novoselov

University of Nebraska-Lincoln, snovoselov2@unl.edu

Dmitri E. Fomenko

University of Nebraska-Lincoln, dfomenko2@unl.edu

See next page for additional authors

Follow this and additional works at: <https://digitalcommons.unl.edu/biochemgladyshev>



Part of the [Biochemistry, Biophysics, and Structural Biology Commons](#)

Sengupta, Aniruddha; Carlson, Bradley A.; Weaver, James A.; Novoselov, Sergey V.; Fomenko, Dmitri E.; Gladyshev, Vadim N.; and Hatfield, Dolph, "A Functional Link between Housekeeping Selenoproteins and Phase II Enzymes" (2008). *Vadim Gladyshev Publications*. 92.

<https://digitalcommons.unl.edu/biochemgladyshev/92>

This Article is brought to you for free and open access by the Biochemistry, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Vadim Gladyshev Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Aniruddha Sengupta, Bradley A. Carlson, James A. Weaver, Sergey V. Novoselov, Dmitri E. Fomenko, Vadim N. Gladyshev, and Dolph Hatfield

A functional link between housekeeping selenoproteins and phase II enzymes

Aniruddha SENGUPTA*, Bradley A. CARLSON*, James A. WEAVER*, Sergey V. NOVOSELOV†, Dmitri E. FOMENKO†, Vadim N. GLADYSHEV† and Dolph L. HATFIELD*¹

*Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, CCR, NCI, NIH, Bethesda, MD 20892, U.S.A., and †Department of Biochemistry, University of Nebraska, Lincoln, NE 68588, U.S.A.

Sec (selenocysteine) is biosynthesized on its tRNA and incorporated into selenium-containing proteins (selenoproteins) as the 21st amino acid residue. Selenoprotein synthesis is dependent on Sec tRNA and the expression of this class of proteins can be modulated by altering Sec tRNA expression. The gene encoding Sec tRNA (*Trsp*) is a single-copy gene and its targeted removal in liver demonstrated that selenoproteins are essential for proper function wherein their absence leads to necrosis and hepatocellular degeneration. In the present study, we found that the complete loss of selenoproteins in liver was compensated

for by an enhanced expression of several phase II response genes and their corresponding gene products. The replacement of selenoprotein synthesis in mice carrying mutant *Trsp* transgenes, wherein housekeeping, but not stress-related selenoproteins are expressed, led to normal expression of phase II response genes. Thus the present study provides evidence for a functional link between housekeeping selenoproteins and phase II enzymes.

Key words: gene expression, liver, microarray, selenocysteine (Sec) tRNA, *Trsp* knockout, xenobiotic.

INTRODUCTION

Several trace elements have important roles in human health and their over-abundance or reduced levels result in severe health problems. Selenium is one such essential micronutrient with antioxidant properties, whose deficiency has been associated with several disorders [1]. Selenium is incorporated into proteins (selenoproteins) as the amino acid Sec (selenocysteine) and its biological function is believed to be exerted in large part by these proteins [2]. To date, 25 selenoprotein genes have been identified in the human genome and 24 in the mouse genome [3]. The incorporation of Sec into proteins is a unique process in that it uses the stop codon, UGA, to decode this amino acid and involves a distinctive tRNA, designated tRNA^{[Ser]Sec}. A number of other *cis*- and *trans*-acting factors are also required that form a complex with Sec-tRNA^{[Ser]Sec} mediating the co-translational incorporation of Sec into protein [2,4,5].

Higher vertebrates have two Sec-tRNA^{[Ser]Sec} isoforms that differ from each other by a single methyl group on the 2'-O-hydroxyribosyl moiety at position 34 [2]. This methyl group is designated Um34. Both isoforms also contain the base, 5'-methylcarboxymethyluracil (mcm⁵U), at position 34. Since both isoforms contain mcm⁵U, but only one of them contains Um34, they are designated mcm⁵U (i.e. the isoform lacking Um34) and 5'-methylcarboxymethyl-2'-O-methyluridine (mcm⁵Um; i.e. the isoform containing Um34). Sec-tRNA^{[Ser]Sec} has three additional modified bases, pseudouridine at position 55, 1-methyladenosine at position 58 and N⁶-isopentenyladenosine (i⁶A) at position 37. The addition of Um34 is the last step in the maturation of Sec-tRNA^{[Ser]Sec} and this step is stringently dependent on the prior synthesis of all base modifications [6]. In addition, Um34 synthesis is influenced by selenium status, and selenium deficiency leads to an enrichment of mcm⁵U as compared

with mcm⁵Um, whereas selenium adequacy reverses this ratio [2]. The levels of the two isoforms modulate expression of different selenoproteins wherein some selenoproteins [e.g. GPx (glutathione peroxidase) 1 and 3 that function largely as stress-related proteins] are preferentially expressed in the presence of mcm⁵Um, whereas others [e.g. TR (thioredoxin reductase) 1 and 3 that function as essential housekeeping proteins] are preferentially expressed in the presence of mcm⁵U [7,8].

The gene encoding Sec-tRNA^{[Ser]Sec} (*Trsp*) is present in single copy and its expression is essential for the synthesis of all selenoproteins. Selenoproteins are the only known class of proteins in eukaryotes whose expression is regulated by a single tRNA, and manipulating the expression of *Trsp* in mice modulates selenoprotein synthesis. Since removal of *Trsp* is embryonic lethal [9,10], the conditional knockout of *Trsp* [10] gave rise to several useful models for studying the role of selenium and selenoproteins in development and health (reviewed in [11]). In one of these models, we targeted the removal of *Trsp* in hepatocytes that demonstrated an essential role of selenoproteins in proper liver function [12]. Additionally, we rescued *Trsp* null mice with transgenic mice carrying a mutant *Trsp* transgene [7,8]. In one mutant *Trsp* transgene, A37 was changed to G [7,13], which resulted in loss of both i⁶A and Um34 [6]. We also produced a second transgenic mouse, where T34 was changed to A in the mutant transgene [8] and the resulting tRNA gene product also lacked Um34. Transgenic mice carrying mutant *Trsp* transgenes were used to replace selenoprotein synthesis in mice lacking *Trsp* in hepatocytes by matings between these two mouse lines as described previously [8]. Introduction of either the A34 or G37 mutant transgenes into the liver *Trsp*-knockout mice selectively replaced selenoproteins involved in housekeeping functions, but not those involved in stress-related functions [7,8]. Furthermore, the number of gene copies of the mutant G37 transgene varied

Abbreviations used: AOX, aldehyde oxidase; EPHX, epoxide hydrolase; GPx, glutathione peroxidase; GST, glutathione transferase; GSTA, GST Alpha; GSTM, GST Mu; HMOX, haem oxygenase; HRP, horseradish peroxidase; i⁶A, N⁶-isopentenyladenosine; mcm⁵U, 5'-methylcarboxymethyluracil; mcm⁵Um, 5'-methylcarboxymethyl-2'-O-methyluridine; Q-PCR, quantitative real-time PCR; ROS, reactive oxygen species; RT, reverse transcriptase; Sec, selenocysteine; TR, thioredoxin reductase; Um34, 2'-O-methylribose at position 34 in selenocysteine tRNA.

¹ To whom correspondence should be addressed (email hatfield@mail.nih.gov).

Table 1 Summary of mouse lines, their genotypes and designations

Mouse	Genotype	Description	Designation (used in the text)
Wild-type	<i>Trsp</i> ^{+/+} - <i>AlbCre</i> ^{+/+}	Homozygous for <i>Trsp</i> and albumin <i>Cre</i>	<i>Trsp</i>
<i>Trsp</i> liver knockout	<i>Trsp</i> ^{fl/fl} - <i>AlbCre</i> ^{+/+}	Lacks <i>Trsp</i> in the liver	Δ <i>Trsp</i>
A34 transgenic (two copies)	<i>Trsp</i> ^{fl/fl} - <i>AlbCre</i> ^{+/+} -A34 ^{+/+}	Carries two copies of the T34 → A34 mutant transgene	A34
G37 transgenic (two copies)	<i>Trsp</i> ^{fl/fl} - <i>AlbCre</i> ^{+/+} -G37 ^{+/+}	Carries two copies of the A37 → G37 mutant transgene	G37L
G37 transgenic (16 copies)	<i>Trsp</i> ^{fl/fl} - <i>AlbCre</i> ^{+/+} -G37 ^{1/1}	Carries 16 copies of the A37 → G37 mutant transgene	G37H

from 2 in one of the transgenic mouse lines we developed to 16 in the other transgenic mouse line.

In the present study, a comparative analysis of gene expression in the liver of the *Trsp*-knockout mice, designated Δ *Trsp* herein, and the A34 and G37 transgenic mice with gene expression of wild-type mice, was carried out using microarrays. These studies showed that the loss of selenoproteins in *Trsp*-knockout mice was associated with an enhanced expression of several phase II response genes and their corresponding enzymes. Phase II response genes are enzymes involved in detoxification as well as protection against oxidative stress. Interestingly, replacement of housekeeping selenoproteins in A34 or G37 transgenic mice resulted in the levels of phase II enzymes returning to normal. Taken together, the results suggest a functional association between housekeeping selenoproteins and phase II enzymes, wherein the loss of function of some housekeeping selenoproteins may be compensated for by phase II enzymes in the liver of the knockout mouse.

EXPERIMENTAL

Materials

NuPage polyacrylamide gels, PVDF membranes, See-Blue Plus2 protein markers, TRIzol[®] and Superscript II RT (reverse transcriptase) were purchased from Invitrogen. SuperSignal West Dura extended duration substrate was from Pierce, and Cy3 and Cy5 mono-reactive dyes were from GE Healthcare. GSTA [GST (glutathione transferase) Alpha], GSTM (GST Mu) and EPHX1 (epoxide hydrolase 1) antibodies were obtained from Detroit R&D, and HMOX1 (haem oxygenase 1) antibodies, anti-mouse and anti-rabbit HRP (horseradish peroxidase)-conjugated secondary antibodies were from Santa Cruz Biotechnology. AOX1 (aldehyde oxidase 1) antibodies were from BD Biosciences and β -actin antibodies and anti-goat HRP-conjugated secondary antibodies were from Abcam. Primers used for real-time PCR were obtained from Sigma-Genosys. All other reagents were of the highest grade available and were obtained commercially.

Mouse lines and genotyping

The mice analysed in the present study were all males, 6–8 weeks of age in a B6/FVB genetic background and were fed a selenium-sufficient diet. Each mouse line used in the present study, preparation of the mutation carried in the A34 and G37 *Trsp* transgenes, and the manner in which these mouse lines were generated are described in detail elsewhere [7,8], and their genotypes and designations are summarized in Table 1. The care of animals was in accordance with the National Institutes of Health institutional guidelines under the expert direction of Dr Kyle Stump (NCI, National Institutes of Health, Bethesda, MD, U.S.A.). DNA was extracted from mouse tail clippings and the genotype determined by PCR with the appropriate primers as described previously [7,12].

Probe preparation

Total RNA from liver of wild-type (*Trsp*), liver knockout (Δ *Trsp*) and transgenic (A34, G37L and G37H; for an explanation of these see Table 1) mice was isolated using TRIzol[®] reagent according to the manufacturer's protocol (Invitrogen) and labelled using the Fairplay[®] II microarray labelling kit (Stratagene). For indirect labelling of RNA, 15 μ g of both control and experimental RNA was used to generate cDNA, using aminoallyl dNTP mix according to the manufacturer's protocol. The resulting cDNA was purified using a MinElute column (Qiagen) and eluted from the column with 10 μ l of elution buffer (provided in the MinElute kit) and dried using a speed-vac for 15 min. Samples were next coupled to 5 μ l of 2 \times coupling buffer (provided in the Fairplay[®] II microarray labelling kit) and 5 μ l of monofunctional dye and incubated at room temperature (22 °C) in the dark for 30 min. Following incubation, the labelled cDNA was purified using a MinElute column and eluted with 10 μ l of elution buffer.

Microarray hybridization

Mouse oligonucleotide glass arrays, containing 70-mer oligonucleotides (printed on Corning epoxide slides), were obtained from the NCI Microarray Facility, Frederick, MD, U.S.A. Each slide in these oligonucleotide arrays has 48 blocks containing 28 rows and 28 columns, each with 36960 oligonucleotide spots with a spacing of 155 μ m.

Slides were pre-hybridized for 1 h at 42 °C with 40 μ l of pre-hybridization buffer [5 \times SSC (1 \times SSC is 0.15 M NaCl/0.015 M sodium citrate), 1 % BSA and 0.1 % SDS]. Pre-hybridization solution was removed by plunging the slides, first into deionized water and then into propan-2-ol, for 2 min each. The slides were air-dried prior to hybridization. For hybridization, the Cy3- and Cy5-labelled cDNAs were combined and mixed with 1 μ l of COT-1 DNA, preheated at 100 °C for 1 min to denature the targets and snap cooled on ice. This mixture was added to 20 μ l of 2 \times F-hybridization buffer (50 % formamide, 10 \times SSC and 0.2 % SDS) and pre-warmed at 42 °C. The total cDNA/hybridization solution mixture was loaded on to each pre-hybridized slide and covered with an M Series Lifterslip (Erie Scientific). The slides were placed in hybridization chambers and incubated overnight at 42 °C. The humidity in each chamber was maintained by the addition of 20 μ l of 3 \times SSC solution. Post-hybridization washing included 5 min in 2 \times SSC + 0.1 % SDS, 5 min in 1 \times SSC and 5 min in 0.2 \times SSC, after which the slides were dried by centrifugation (44 g for 5 min at 22 °C).

Data processing and analysis

Microarray slides were scanned for each fluoroprobe at 10 μ m using a Genepix[®] 4000B scanner and analysed with GenePix Pro 3.0 software (Axon Instruments). Scanned images were exported as Tiff files to GenePix Pro 3.0 software for analysis. For data analysis, data files (in gpr format) and images (in jpeg format) were imported into the microarray database (mAdb)

Table 2 Primers for assessing real-time PCR

Primers designed for determining real-time PCR of each mRNA examined are shown.

Gene	Forward sequence	Reverse sequence
<i>Aox1</i>	5'-GAAGCTGGACAACGCTTACA-3'	5'-CCACATTTGATTGCCACTTC-3'
<i>Cd36</i>	5'-GATTGTACCTGGGAGTTGGC-3'	5'-CATGAGAATGCCTCAAACA-3'
<i>Ces1</i>	5'-CAGAAGACAGCTGCATCCAT-3'	5'-TCCAATCAAGTCCAGGAACA-3'
<i>Ces2</i>	5'-ATGTGAGGCTATGGATTCCC-3'	5'-TCCTCAGATGCCAACAACTC-3'
<i>Cyp2A5</i>	5'-GAGATTGATCGGGTGATTGG-3'	5'-CGAACTTGGTGTCTTGGT-3'
<i>Ddc</i>	5'-CTGAATGGTGTGGAGTTGC-3'	5'-TGAATCCTGAGTCTGGTGA-3'
<i>Dmpk</i>	5'-CGTGTTCCGCTATGAGATG-3'	5'-ACGAATGAGGTCCTGAGCTT-3'
<i>Ephx1</i>	5'-GGGTCAAAGCCATCAGCCA-3'	5'-CCTCCAGAAGGACACCACTTT-3'
<i>Gsta1</i>	5'-CGCAGACCAAGGCCATTCTC-3'	5'-TTGCCAATCAITTCAGTCAGA-3'
<i>Gsta2</i>	5'-CCCCTTTCCCTCTGCTGAAG-3'	5'-TGCAGCCACACTAAACTTGA-3'
<i>Gsta4</i>	5'-TTGAAATCGATGGGATGATG-3'	5'-ATCATCATCAGGTCCTGGGT-3'
<i>Gstm1</i>	5'-CCAAACACAGGAGGTACGCC-3'	5'-CGTCACCCATGGGTGATCTC-3'
<i>Gstm2</i>	5'-CCTATGACACTAGGTACTGG-3'	5'-CACTGGCTTCGGTCCATGCA-3'
<i>Gstm3</i>	5'-TATGACACTGGGCTATTGGAAC-3'	5'-GGGCATCCCCCATGACA-3'
<i>Gstt3</i>	5'-GGCAGAAGATGATTTCCCT-3'	5'-TCAGCCACAGAAATATGGGA-3'
<i>Hmx1</i>	5'-GCCACCAAGGAGGTACACAT-3'	5'-GCTTGTGGCGCTCTATCTCC-3'
<i>Htatip2</i>	5'-GGCCAGGAGTCTACTGTGT-3'	5'-GTTCAGCATCGCTCAACCA-3'
<i>Ikbbk</i>	5'-CCTGGTAGCCAAACAGGAAT-3'	5'-CCTTCTCTCCACCAGCTTC-3'
<i>Lgals1</i>	5'-GCAACAACCTGTGCCTACAC-3'	5'-TGATGCACACCTCTGTGATG-3'
<i>Srxn1</i>	5'-CCAGGGTGGCGACTACTACT-3'	5'-CAAGTCTGGTGTGGATGCTC-3'
<i>Ugdh</i>	5'-TGCTGTCCAATCCTGAGTTC-3'	5'-ACCCAGTGCTCATACACAGC-3'
<i>Ugt2b35</i>	5'-AATGACCTTCTCGGTCATCC-3'	5'-CCACCATGTGTGAATGTTA-3'

and analysed using software tools provided by the National Cancer Institute, Center for Cancer Research in collaboration with the National Institutes of Health, Center for Information Technology, Bioinformatics and Molecular Analysis Section. Transcripts whose expression level varied at least 2-fold in $\Delta Trsp$ mice as compared with *Trsp* mice in more than 50 % of the experiments with a P value ≤ 0.05 were selected and the corresponding transcript levels were then analysed in A34, G37L and G37H transgenic mice relative to *Trsp* mice. A hierarchical clustering analysis was performed on genes in the resultant analysis. Grouping of genes into different biological functions was performed using the David database (<http://david.abcc.ncifcrf.gov>) and/or the mAdb software.

Q-PCR (quantitative real-time PCR)

Two-step Q-PCR was performed to validate the relative expression of genes, using the primer sequences outlined in Table 2. For each sample, 2 μ g of total RNA was reverse transcribed to synthesize first strand cDNA using SuperScript II RT enzyme and random primers. The resulting cDNA was diluted, and in combination with 500 nM of each primer, iQTM SYBR green supermix (Bio-Rad Laboratories) and DNA Engine Opticon[®] 2 Real-Time PCR Detection System (MJ Research), used for transcript quantification. The PCR reaction had an initial denaturation of 5 min at 95 °C, followed by 40 cycles consisting of 20 s at 94 °C, 20 s at 55 °C and 30 s at 72 °C. The reactions were carried out in triplicate and the specificity of the primers was verified by melting curve analysis. RNA levels were normalized to β -glucuronidase (*Gusb*) and expression levels were compared with those of wild-type mice.

Western blotting

Protein extracts prepared from liver of *Trsp*, $\Delta Trsp$, A34, G37L and G37H mice were electrophoresed on 10 % polyacrylamide gels, transferred on to PVDF membranes and immunoblotted with antibodies against GSTA (1:10 000 dilution), GSTM (1:10 000 dilution), EPHX1 (1:10 000 dilution), HMOX1 (1:500 dilution),

AOX1 (1:250 dilution) and β -actin (1:1000 dilution). Anti-goat HRP-conjugated secondary antibody (1:40 000) was used for GSTA, GSTM, EPHX1 and β -actin, whereas anti-rabbit HRP-conjugated secondary antibody (1:25 000) was used for HMOX1, and anti-mouse HRP-conjugated secondary antibody (1:30 000) was used for AOX1. Following the attachment of the secondary antibody, membranes were washed with TBS (Tris-buffered saline, 20 mM Tris/HCl, pH 7.5 and 150 mM NaCl) containing 0.1 % Tween 20, incubated in SuperSignal West Dura Extended Duration Substrate and exposed to X-ray film.

RESULTS

Gene expression profile in liver of $\Delta Trsp$ mice

The overall gene expression profile associated with the conditional knockout of *Trsp* in mouse liver ($\Delta Trsp$) and in liver of mice following the selective replacement of selenoproteins with mutated *Trsp* transgenes was examined (Figure 1). An analysis of gene expression in livers from *Trsp*, $\Delta Trsp$, A34, G37L and G37H mice showed that the loss of *Trsp* was associated with altered levels of some mRNAs, reflected through changes in gene expression and/or mRNA stability. Initially, gene expression in $\Delta Trsp$ mice was compared with that in *Trsp* mice and genes displaying a greater than 2-fold change in the microarray analysis with a P value ≤ 0.05 were selected. These genes were then segregated as up-regulated (Table 3) or down-regulated (Table 4) and ordered by their pattern of gene expression by hierarchical clustering (Figure 1). Transcripts up-regulated in $\Delta Trsp$ mice are shown in Figure 1(A) and those down-regulated are shown in Figure 1(B), along with the relative expression of these transcripts in A34, G37L and G37H transgenic mice. Following filtering, genes up-regulated in $\Delta Trsp$ mice were grouped under six major hierarchical clusters, whereas those down-regulated were grouped into five major hierarchical clusters.

Genes elevated or repressed in $\Delta Trsp$ mice in comparison with *Trsp* mice were grouped according to their functions, whereas the transcript levels of corresponding genes in A34, G37L and G37H transgenic mice were analysed relative to *Trsp* and are represented in Tables 3 and 4. Genes elevated in $\Delta Trsp$ (Table 3) were mainly involved in detoxification, stress response, xenobiotic metabolism, intracellular communication, cellular transport and cell growth and differentiation. Some genes that were significantly up-regulated in $\Delta Trsp$ mice are involved in detoxification and xenobiotic metabolism and include epoxide hydrolase 1 (*Ephx1*), carboxylesterase 1 and 2 (*Ces1*, *Ces2*), cytochrome P450, family 2, subfamily a, polypeptide 5 (*Cyp2a5*), members of the glutathione transferase family (*Gst*), haem oxygenase 1 (*Hmx1*) and aldehyde oxidase 1 (*Aox1*). Interestingly, the levels of expression of these genes in A34, G37L and G37H mice were similar to *Trsp*.

Genes that were repressed in $\Delta Trsp$ mice compared with *Trsp* mice were grouped in a similar manner to those manifesting enhanced expression and their relative transcript levels were measured in transgenic mice (Table 4). Although most of the genes down-regulated in $\Delta Trsp$ mice had diverse or unknown functions, some of them could be grouped as being involved in transcription, intracellular communication and cellular transport.

Q-PCR validation of elevated genes

The expression levels of 22 genes elevated in $\Delta Trsp$ mice were verified by Q-PCR (Figure 2) and were in excellent agreement with the microarray analysis. Expression of the corresponding

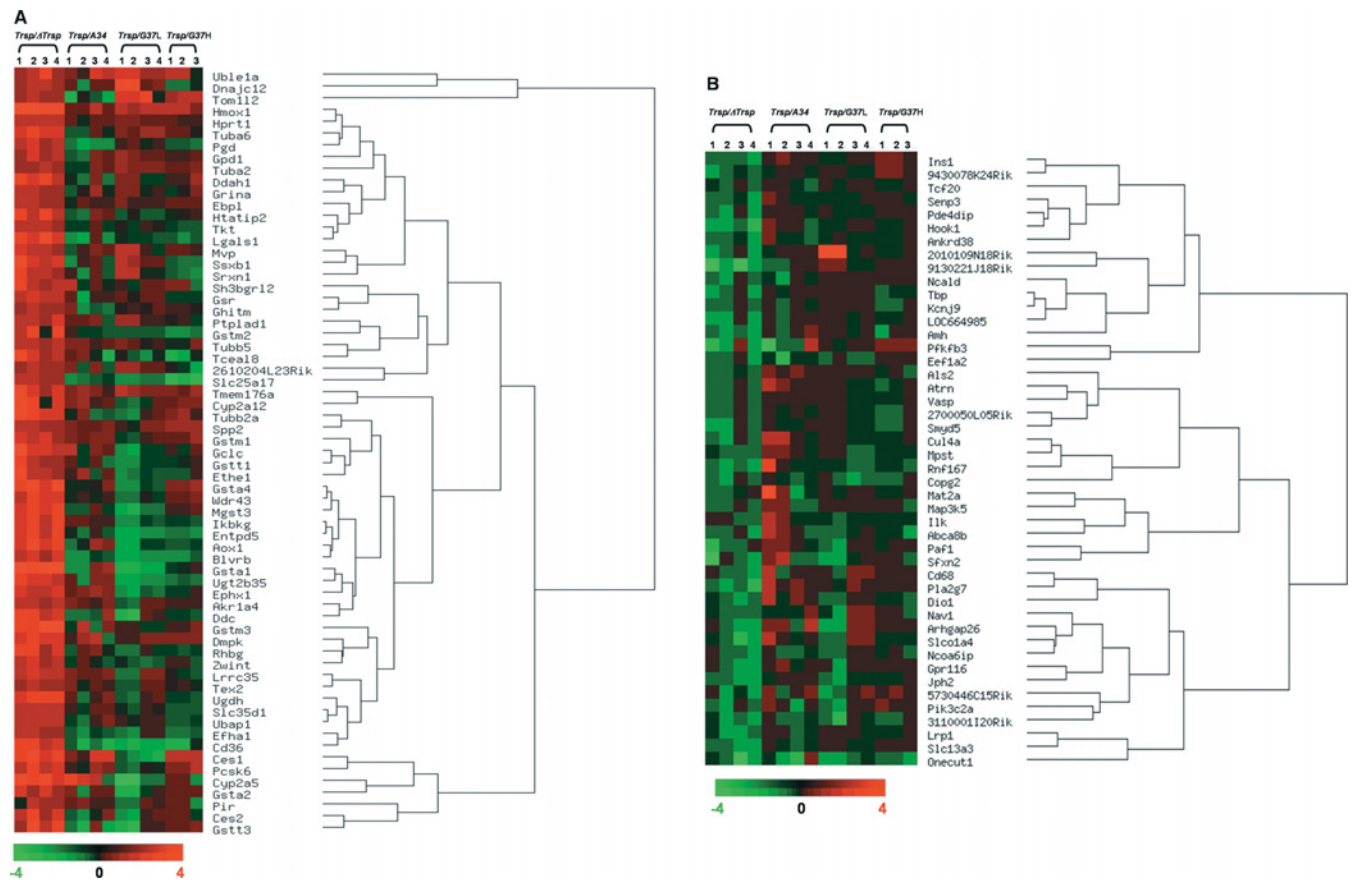


Figure 1 Hierarchical clustering analysis of gene alterations following *Trsp* removal in liver

Hierarchical dendrogram representing the expression profiles of significantly altered genes, following data filtering as described in the Results section, (A) up-regulated or (B) down-regulated in knockout mice. The genes are ordered by clustering tightness, with a distance measure of 1 (Pearson correlation coefficient) and a *P* value threshold of 0.05. Each column represents data from one experimental set, and rows indicate individual genes. Increases and decreases in transcript expression levels are represented by shades of red and green respectively.

transcripts in transgenic mice was similar to that in *Trsp* mice. The genes analysed by Q-PCR were grouped according to their function (Table 3) as metabolism (Figure 2A), defence stress and detoxification (Figure 2B), intracellular communication/signal transduction (Figure 2C) and cell cycle/growth and differentiation (Figure 2D).

Expression of phase II enzymes

Protein expression profiles from the five mouse lines appeared similar in liver samples, as observed on Coomassie-Blue-stained gels, with the exception of a prominently enriched band of approx. 25 kDa in $\Delta Trsp$ mice (Figure 3A, indicated by the arrow). This observation was also noted in a previous study and the elevated band was sequenced and identified as GST [12]. As expected, the mRNA levels of the *Gst* isoforms were also increased (Figure 2). To verify that the induced mRNA levels also gave rise to a consequential increase in the corresponding protein levels, we analysed the amounts of several phase II enzymes by Western blotting (Figure 3B). Indeed, a marked increase in two of the GST isoforms, GSTA and GSTM, was observed in $\Delta Trsp$ mice as compared with *Trsp*. Furthermore, several other Phase II proteins, EPHX1, HMOX1 and AOX1, were increased in $\Delta Trsp$ mice compared with *Trsp*. The increase in the amounts of these proteins in $\Delta Trsp$ mice paralleled their induced mRNA levels. Most interestingly, the protein levels of these enzymes in

the transgenic mice were virtually the same as in *Trsp* mice, providing strong evidence that the link between enhanced Phase II protein expression and loss of selenoprotein expression is due to the absence of one or more housekeeping selenoproteins. These observations are further considered below. β -Actin was examined by Western blotting as a control protein and its level was unaffected in the five mouse lines (see lowest panel in Figure 3B).

DISCUSSION

Biochemical and *in silico* studies have identified 25 selenoprotein genes in humans and 24 in mice [3,14]. The functions of many of these selenoproteins have not been identified and most that have been characterized serve as oxidoreductases associated with various metabolic pathways, e.g. free radical scavenging, maintenance of intracellular redox status and repair of oxidized methionine residues [15,16]. Our previous studies have shown that selective knockout of *Trsp* in mouse hepatocytes resulted in the virtual absence of selenoproteins in liver and a pronounced reduction in selenium levels, even though the low molecular mass selenocompounds were little affected [12]. These results demonstrated that selenoproteins are essential for proper liver function and their absence causes severe necrosis and hepatocellular degeneration, accompanied by necrosis of peritoneal and retroperitoneal fat

Table 3 Transcripts at elevated levels within liver of knockout mice relative to wild type mice

Genes elevated ≥ 2 -fold in $\Delta Trsp$ mice compared with *Trsp* mice with a *P* value ≤ 0.05 were assessed and are shown in the Table. The corresponding transcript levels were also analysed in A34, G37L and G37H replacement mice relative to *Trsp* mice. Gene Unigene Accession Number, gene description, fold change with *P* value and gene function(s) are shown. Four control and four experimental animals were used for comparing $\Delta Trsp$, A34 and G37L with *Trsp*, whereas three control and three experimental animals were used for comparing G37H with *Trsp*. Genes are grouped into classes according to function.

UniGene	Description	$\Delta Trsp$ compared with <i>Trsp</i>		A34 compared with <i>Trsp</i>		G37L compared with <i>Trsp</i>		G37H compared with <i>Trsp</i>		Function/gene ontology
		Fold change	P value	Fold change	P value	Fold change	P value	Fold change	P value	
Defence stress and detoxification										
Mm.28191	Carboxylesterase 2 (<i>Ces2</i>)	3.8	0.02	−0.7	0.07	−0.5	0.17	0.5	0.53	Ester hydrolase activity
Mm.389848	Cytochrome P450, family 2, subfamily a, polypeptide 5 (<i>Cyp2a5</i>)	8.2	0.01	3.0	0.30	−5.6	0.03	0.2	0.51	Degradation of environmental toxins and mutagens
Mm.32550	DnaJ [Hsp (heat-shock protein) 40] homologue, subfamily C, member 12 (<i>Dnajc12</i>)	2.2	0.00	0.4	0.51	5.5	0.11	−1.5	0.00	Heat-shock protein binding, protein folding
Mm.218639	Sulfiredoxin 1 homologue (<i>Saccharomyces cerevisiae</i>) (<i>Srxn1</i>)	3.3	0.03	−0.2	0.19	0.9	0.96	−1.6	0.00	Antioxidant activity, response to oxidative stress
Metabolism										
Mm.30085	Aldo-keto reductase family 1, member A4 (aldehyde reductase) (<i>Akr1a4</i>)	2.4	0.00	1.3	0.03	−0.1	0.18	1.1	0.01	Glycerolipid metabolism
Mm.26787	Aldehyde oxidase 1 (<i>Aox1</i>)	6.5	0.01	0.7	0.73	−1.8	0.00	−1.1	0.00	Xenobiotic metabolism
Mm.24021	Biliverdin reductase B (<i>Blvrb</i>)	3.4	0.01	−1.5	0.00	−2.1	0.00	−1.7	0.00	Porphyrin and chlorophyll metabolism
Mm.22720	Carboxylesterase 1 (<i>Ces1</i>)	8.0	0.01	2.8	0.09	−2.4	0.00	3.0	0.00	Alkaloid biosynthesis
Mm.12906	Dopa decarboxylase (<i>Ddc</i>)	5.1	0.00	−0.5	0.15	−2.0	0.15	−1.1	0.00	Amino acid and derivative metabolic process
Mm.22758	Emopamil binding protein-like (<i>Ebpl</i>)	2.2	0.00	0.6	0.48	1.2	0.09	1.3	0.00	Sterol metabolism
Mm.10211	Ectonucleoside triphosphate diphosphohydrolase 5, transcript variant 1 (<i>Entpd5</i>)	5.4	0.02	−0.7	0.03	−2.3	0.00	−1.6	0.00	Purine metabolism; pyrimidine metabolism
Mm.9075	Epoxide hydrolase 1, microsomal (<i>Ephx1</i>)	3.3	0.00	1.5	0.02	−1.8	0.00	−0.3	0.14	Xenobiotic metabolism
Mm.252391	Glycerol-3-phosphate dehydrogenase 1 (soluble) (<i>Gpd1</i>)	3.1	0.02	−0.1	0.24	1.4	0.02	1.2	0.08	Carbohydrate metabolism
Mm.283573	Glutathione reductase 1 (<i>Gsr</i>)	3.2	0.00	0.2	0.42	1.2	0.02	−1.1	0.00	Glutathione metabolism
Mm.197422	GST, Alpha 1 (Ya) (<i>Gsta1</i>)	23.4	0.00	−0.2	0.33	−3.6	0.00	−0.3	0.14	Glutathione metabolism; xenobiotic metabolism
Mm.422778	GST, Alpha 2 (Yc2) (<i>Gsta2</i>)	2.9	0.001	0.45	0.55	−2.3	0.06	1.2	0.00	Glutathione metabolism; xenobiotic metabolism
Mm.2662	GST, Alpha 4 (<i>Gsta4</i>)	8.6	0.00	0.6	0.51	−1.5	0.00	1.8	0.05	Glutathione metabolism; xenobiotic metabolism
Mm.37199	GST, Mu 1 (<i>Gstm1</i>)	3.2	0.01	1.4	0.00	0.0	0.20	1.5	0.10	Glutathione metabolism; xenobiotic metabolism
Mm.37199	GST, Mu 2 (<i>Gstm2</i>)	4.8	0.01	−0.6	0.03	−1.2	0.00	−1.8	0.00	Glutathione metabolism; xenobiotic metabolism
Mm.37199	GST, Mu 3 (<i>Gstm3</i>)	12.0	0.01	0.4	0.54	0.0	0.16	1.2	0.21	Glutathione metabolism; xenobiotic metabolism
Mm.5731	GST, Theta 3 (<i>Gstt3</i>)	5.1	0.04	−1.8	0.07	−2.4	0.15	1.3	0.01	Glutathione metabolism
Mm.276389	Haem oxygenase (decycling) 1 (<i>Hmox1</i>)	17.3	0.00	1.6	0.08	2.4	0.00	2.2	0.02	Porphyrin and chlorophyll metabolism; xenobiotic metabolism
Mm.299381	Hypoxanthine guanine phosphoribosyl transferase 1 (<i>Hprt1</i>)	2.8	0.00	0.6	0.54	1.3	0.00	1.1	0.12	Purine metabolism
Mm.218286	Microsomal GST 3 (<i>Mgst3</i>)	5.8	0.00	−0.5	0.05	−2.7	0.00	1.1	0.04	Glutathione metabolism; xenobiotic metabolism
Mm.344831	UDP-glucose dehydrogenase (<i>Ugdh</i>)	6.4	0.002	0.5	0.56	−0.1	0.16	−1.2	0.00	Nucleotide sugar metabolism; starch and sucrose metabolism
Mm.312095	UDP glucuronosyltransferase 2 family, polypeptide B35 (<i>Ugt2b35</i>)	5.0	0.01	0.6	0.61	−2.4	0.00	−1.4	0.00	Sugar metabolism
Intracellular communication/signal transduction										
Mm.18628	CD36 antigen (<i>Cd36</i>)	5.6	0.00	−2.6	0.00	−3.2	0.00	−3.8	0.00	Adipocytokine signalling pathway; PPAR signalling pathway
Mm.6529	Dystrophia myotonica-protein kinase (<i>Dmpk</i>)	5.0	0.00	1.0	0.97	1.3	0.06	1.5	0.00	Protein amino acid phosphorylation; regulation of small GTPase-mediated signal transduction
Mm.12967	Inhibitor of κ B kinase γ , transcript variant 2 (<i>Ikbkg</i>)	5.1	0.02	1.2	0.01	−1.6	0.00	−1.1	0.00	Activation of NF- κ B (nuclear factor κ B)-inducing kinase

Table 3 (Cont.)

UniGene	Description	Δ <i>Trsp</i> compared with <i>Trsp</i>		A34 compared with <i>Trsp</i>		G37L compared with <i>Trsp</i>		G37H compared with <i>Trsp</i>		Function/gene ontology
		Fold change	P value	Fold change	P value	Fold change	P value	Fold change	P value	
Mm.294007	Predicted: proprotein convertase subtilisin/kexin type 6, transcript variant 4 (<i>Pcsk6</i>)	3.7	0.00	0.9	0.94	−0.7	0.03	1.8	0.04	Determination of left/right symmetry; transmembrane receptor protein tyrosine kinase signalling pathway
Mm.308180	Protein tyrosine phosphatase-like A domain containing 1 (<i>Ptplad1</i>)	3.0	0.00	0.7	0.64	1.6	0.09	0.3	0.41	I κ B (inhibitor of NF- κ B) kinase/NF- κ B cascade; JNK (c-Jun N-terminal kinase) cascade; Rho protein signal transduction
Cell cycle/growth and differentiation										
Mm.20801	HIV-1 tat interactive protein 2, homologue (human) (<i>Htatip2</i>)	4.2	0.01	−0.1	0.22	−0.7	0.03	0.4	0.40	Regulation of angiogenesis and apoptosis; cell differentiation
Mm.43831	Lectin, galactose binding, soluble 1 (<i>Lgals1</i>)	6.0	0.01	−1.4	0.03	−1.1	0.00	−1.5	0.00	Myoblast differentiation; sugar binding
Mm.62876	ZW10 interactor (<i>Zwint</i>)	3.0	0.01	−0.5	0.04	−1.2	0.00	0.3	0.43	cell cycle; cell division
Mm.290692	Transketolase (<i>Tkt</i>)	2.6	0.00	−0.5	0.03	0.5	0.40	−1.2	0.00	Metal ion binding; regulation of growth
Cellular transport and transport mechanism										
Mm.103777	Rhesus blood group-associated B glycoprotein (<i>Rhbg</i>)	4.8	0.02	0.1	0.24	−0.8	0.03	−1.1	0.00	Ammonium transporter activity
Mm.222536	Solute carrier family 25 (mitochondrial carrier, peroxisomal membrane protein), member 17 (<i>Slc25a17</i>)	2.4	0.00	−0.9	0.02	−1.9	0.00	−5.8	0.00	Mitochondrial transport; transporter activity
Mm.281800	Solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1 (<i>Slc35d1</i>)	2.8	0.00	0.6	0.53	0.5	0.37	−1.2	0.00	Nucleotide-sugar transporter
Mm.218875	Target of myb1-like 2 (chicken), transcript variant 1 (<i>Tom12</i>)	2.1	0.00	−2.1	0.06	5.4	0.00	2.5	0.01	Golgi vesicle-mediated transport; intracellular protein transport
Transcription/translation/protein modification										
Mm.298030	Synovial sarcoma, X member B, breakpoint 1 (<i>Ssxb1</i>)	5.1	0.02	−0.2	0.20	1.7	0.05	−1.4	0.00	Regulation of transcription
Mm.34483	Leucine-rich repeat containing 35 (<i>Lrrc35</i>)	3.2	0.00	1.4	0.03	−0.1	0.25	−0.4	0.12	Metal-ion binding; protein modification
Miscellaneous (mixed functions)										
Mm.234247	Dimethylarginine dimethylaminohydrolase 1 (<i>Ddah1</i>)	4.5	0.01	−0.6	0.08	0.2	0.33	1.4	0.28	NO biosynthesis; protein amino acid nitrosylation
Mm.26834	EF hand domain family A1 (<i>Etha1</i>)	2.4	0.00	−1.3	0.00	−0.4	0.15	−1.3	0.00	Calcium-ion binding
Mm.182912	Growth hormone inducible transmembrane protein (<i>Ghitm</i>)	2.2	0.00	−0.8	0.07	1.1	0.09	1.1	0.10	Interacts with FtsH
Mm.41665	Glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1 (glutamate binding) (<i>Grina</i>)	2.3	0.00	0.1	0.29	0.7	0.69	0.5	0.54	Receptor activity
Mm.228797	Major vault protein (<i>Mvp</i>)	3.2	0.01	0.6	0.57	1.5	0.62	1.1	0.00	Calcium-ion binding; ribonucleoprotein complex
Mm.252080	Predicted: phosphogluconate dehydrogenase, transcript variant 1 (<i>Pgd</i>)	3.0	0.00	−2.0	0.00	0.5	0.45	−1.2	0.00	Pentose-phosphate shunt, oxidative branch
Mm.293463	Pirin (<i>Pir</i>)	2.2	0.05	1.2	0.01	−0.1	0.18	0.2	0.46	Metal-ion binding
Mm.173058	Secreted phosphoprotein 2 (<i>Spp2</i>)	2.3	0.00	1.3	0.01	0.8	0.74	1.7	0.00	Bone remodelling
Unknown										
Mm.22109	RIKEN cDNA 2610204L23 gene (2610204L23Rik)	2.3	0.00	0.2	0.34	1.6	0.01	−0.8	0.14	Unknown
Mm.100125	SH3 domain binding glutamic acid-rich protein like 2 (<i>Sh3bgrl2</i>)	3.5	0.03	0.8	0.85	0.6	0.55	0.5	0.62	Unknown
Mm.102407	Testis-expressed gene 2 (<i>Tex2</i>)	2.4	0.00	0.7	0.61	0.0	0.20	0.3	0.39	Unknown
Mm.289795	Ubiquitin-associated protein 1 (<i>Ubap1</i>)	2.6	0.00	0.5	0.44	0.6	0.43	−1.2	0.00	Unknown
Mm.257762	Predicted: WD repeat domain 43, transcript variant 9 (<i>Wdr43</i>)	4.1	0.00	0.6	0.43	−0.8	0.03	1.3	0.04	Unknown

Table 4 Transcripts in reduced levels within liver of knockout mice relative to wild-type mice

Genes depleted ≥ 2 -fold in $\Delta Trsp$ mice as compared with *Trsp* mice with a *P* value ≤ 0.05 were assessed and are shown in the Table. The corresponding transcript levels were also analysed in A34, G37L and G37H replacement mice relative to *Trsp* mice. Gene Unigene Accession Number, gene description, fold change with *P* value and gene function(s) are shown. Four control and four experimental animals were used for comparing $\Delta Trsp$, A34 and G37L with *Trsp*, whereas three control and three experimental animals were used for comparing G37H with *Trsp*. Genes are grouped into classes according to function.

UniGene	Description	$\Delta Trsp$ compared with <i>Trsp</i>		A34 compared with <i>Trsp</i>		G37L compared with <i>Trsp</i>		G37H compared with <i>Trsp</i>		Function/gene ontology
		Fold change	<i>P</i> value	Fold change	<i>P</i> value	Fold change	<i>P</i> value	Fold change	<i>P</i> value	
Cellular transport and transport mechanism										
Mm.381860	Coatomer protein complex, subunit γ 2 (<i>Copg2</i>)	−2.3	0.00	−0.8	0.05	−2.5	0.00	−1.7	0.00	Protein transport
Mm.261168	Potassium inwardly rectifying channel, subfamily J, member 9 (<i>Kcnj9</i>)	−2.3	0.00	−0.5	0.08	1.1	0.01	−1.7	0.00	Ion transport; voltage-gated ion channel activity
Mm.283370	Neurocalcin δ (<i>Ncald</i>)	−2.3	0.00	−1.5	0.00	1.5	0.00	1.2	0.00	Vesicle-mediated transport
Mm.296837	Sideroflexin 2 (<i>Sfxn2</i>)	−3.8	0.01	0.1	0.49	−1.4	0.05	0.0	0.43	Ion transport
Mm.250738	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3 (<i>Slc13a3</i>)	−2.4	0.00	−0.8	0.04	−1.2	0.00	1.0	0.00	Dicarboxylic acid transport; ion transport; symporter activity;
Mm.255586	Solute carrier organic anion transporter family, member 1a4 (<i>Slco1a4</i>)	−2.5	0.00	1.0	0.98	−0.3	0.20	−1.2	0.00	Ion transport; organic anion transporter activity
Intracellular communication/signal transduction										
Mm.376094	Anti-Mullerian hormone (<i>Amh</i>)	−2.3	0.00	−0.0	0.41	−0.1	0.24	−1.0	0.32	Transforming growth factor β receptor binding
Mm.271854	Low-density lipoprotein receptor-related protein 1 (<i>Lrp1</i>)	−2.3	0.00	−0.1	0.23	0.0	0.18	−1.3	0.00	Negative regulation of Wnt receptor signalling pathway
Mm.6595	MAPK (mitogen activated protein kinase) kinase kinase 5 (<i>Map3k5</i>)	−2.3	0.00	−0.2	0.35	1.2	0.00	0.1	0.43	Activation of MAPK activity
Mm.3810	Phosphatidylinositol 3-kinase, C2 domain containing, α polypeptide (<i>Pik3c2a</i>)	−2.2	0.00	0.4	0.58	−0.7	0.23	1.6	0.00	Intracellular signalling cascade
Metabolism										
Mm.46269	Insulin I (<i>Ins1</i>)	−2.3	0.00	1.7	0.02	−1.1	0.00	2.1	0.01	Carbohydrate metabolism
Mm.34459	Junctophilin 2 (<i>Jph2</i>)	−2.7	0.00	0.3	0.34	−1.8	0.00	−1.2	0.00	Carbohydrate metabolism
Mm.19669	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (<i>Pfkfb3</i>)	−2.3	0.05	−2.3	0.19	0.7	0.60	2.1	0.01	Fructose and mannose metabolism
Mm.9277	Phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma) (<i>Pla2g7</i>)	−2.8	0.00	2.2	0.14	0.7	0.60	1.1	0.03	Lipid catabolism
Cellular structural organization										
Mm.49994	Hook homologue 1 (<i>Drosophila</i>) (<i>Hook1</i>)	−3.1	0.00	0.4	0.59	0.0	0.19	−1.1	0.00	Cytoskeleton organization and biogenesis; spermatid development
Mm.129840	Phosphodiesterase 4D interacting protein transcript variant 1 (<i>Pde4dip</i>),	−2.4	0.00	0.5	0.56	−1.2	0.00	1.0	0.21	Association with spindle pole body microtubules
Mm.9684	Vasodilator-stimulated phosphoprotein (<i>Vasp</i>)	−2.0	0.00	1.2	0.01	−1.2	0.00	−1.1	0.00	Actin cytoskeleton organization and biogenesis

Table 4 (Cont.)

UniGene	Description	Δ <i>Trsp</i> compared with <i>Trsp</i>		A34 compared with <i>Trsp</i>		G37L compared with <i>Trsp</i>		G37H compared with <i>Trsp</i>		Function/gene ontology
		Fold change	P value	Fold change	P value	Fold change	P value	Fold change	P value	
Transcription/translation										
Mm.174044	RIKEN cDNA 2700050L05 gene, transcript variant 2 (2700050L05Rik)	−2.0	0.00	−1.2	0.00	−1.4	0.00	−1.8	0.00	Transcription regulation
Mm.2645	Eukaryotic translation elongation factor 1 α 2 (<i>Eef1a2</i>)	−2.4	0.00	−2.4	0.02	−1.8	0.00	1.3	0.03	Protein biosynthesis; translational elongation
Mm.303355	One cut domain, family member 1 (<i>Onecut1</i>)	−4.0	0.04	−1.8	0.09	−2.1	0.00	−2.5	0.00	Regulation of transcription
Mm.7916	Paf1, RNA polymerase II associated factor, homologue (<i>S. cerevisiae</i>) (<i>Paf1</i>)	−2.7	0.00	0.7	0.77	−0.9	0.16	0.0	0.31	Regulation of transcription
Mm.244820	TATA box binding protein (<i>Tbp</i>)	−2.6	0.00	0.4	0.45	1.2	0.00	−1.4	0.00	Regulation of transcription
Miscellaneous (mixed functions)										
Mm.28796	RIKEN cDNA 5730446C15 gene, (5730446C15Rik)	−2.1	0.00	0.7	0.74	−0.5	0.24	1.7	0.00	Peptidase activity
Mm.71924	Ankyrin repeat domain 38 (<i>Ankrd38</i>)	−2.3	0.00	−0.7	0.01	−1.3	0.00	−1.1	0.00	Protein–protein interaction
Mm.119936	Attractin (<i>Atrn</i>)	−2.0	0.00	2.2	0.01	−1.1	0.00	−1.7	0.00	Inflammatory response; protein binding; sugar binding
Mm.212861	Cullin 4A (<i>Cul4a</i>)	−2.2	0.00	1.7	0.62	−1.4	0.00	−1.1	0.00	Cell cycle; induction of apoptosis by intracellular signals; ubiquitin cycle
Mm.171323	Nuclear receptor coactivator 6 interacting protein (<i>Ncoa6ip</i>)	−2.4	0.00	−0.8	0.03	−0.4	0.17	−0.6	0.14	S-adenosylmethionine-dependent methyltransferase activity; protein binding
Mm.261818	Ring finger protein 167 (<i>Rnf167</i>)	−2.8	0.00	1.2	0.91	−1.5	0.00	−1.5	0.00	Metal-ion binding; protein binding; peptidase activity
Mm.284592	SUMO/sentrin specific peptidase 3 (<i>Senp3</i>)	−2.2	0.00	0.2	0.35	−0.6	0.04	0.3	0.43	Peptidase activity; protein metabolism; ubiquitin-specific protease activity
Unknown										
Mm.416885	RIKEN cDNA 2010109N18 gene	−2.9	0.00	0.3	0.36	3.5	0.27	−1.4	0.00	Unknown
Mm.138091	Predicted: RIKEN cDNA 3110001I20 gene, transcript variant 1 (3110001I20Rik)	−2.3	0.00	−0.9	0.05	−1.7	0.10	−0.9	0.13	Unknown
Mm.395958	RIKEN cDNA 9130221J18 gene	−4.9	0.00	−0.3	0.14	0.3	0.52	1.4	0.01	Unknown
Mm.395042	RIKEN cDNA 9430078K24 gene	−2.1	0.00	−0.3	0.13	−1.2	0.00	1.8	0.00	Unknown
Mm.425114	Predicted: similar to gonadotropin inducible ovarian transcription factor 1, transcript variant 3 (LOC664985)	−2.9	0.00	0.2	0.35	0.1	0.24	−1.2	0.00	Unknown
Mm.219946	SET and MYND domain containing 5 (<i>Smyd5</i>)	−2.7	0.00	0.0	0.21	−1.1	0.00	−2.0	0.00	Unknown

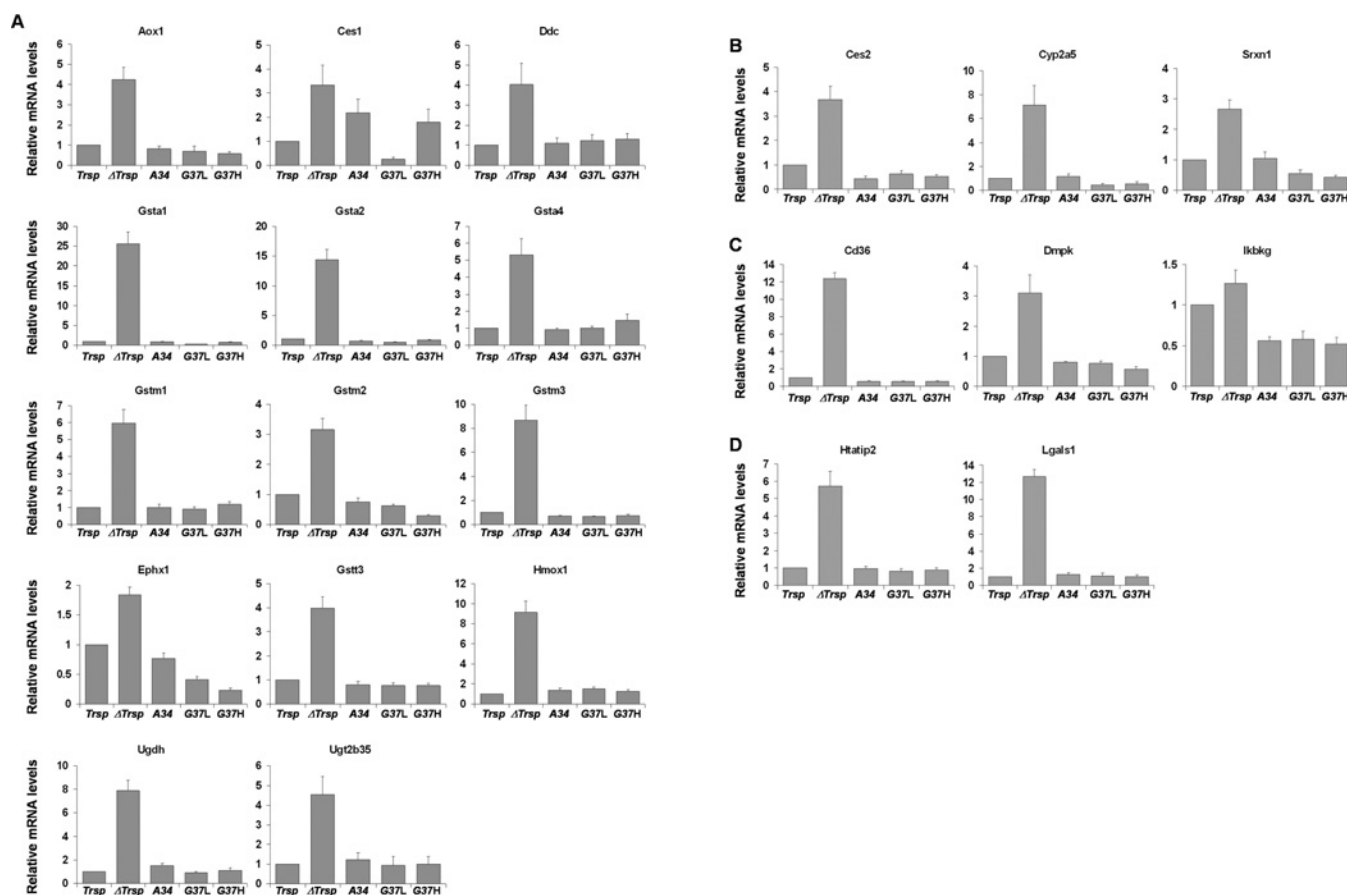


Figure 2 Q-PCR of up-regulated genes following *Trsp* removal in liver

The relative expression of genes up-regulated in $\Delta Trsp$ mice (Table 3) was examined by Q-PCR and normalized to the expression of *Gusb* as described in the Experimental section. The normalized value for each mRNA in liver of $\Delta Trsp$, A34, G37L and G37H mice was then compared with *Trsp* mice and plotted along with error bars. The analysed up-regulated genes are grouped according to their function (see Table 3): (A) metabolism, (B) defense stress and detoxification, (C) intracellular communication/signal transduction, and (D) cell cycle/growth and differentiation. Results represent 3–4 independent experiments, each carried out in triplicate.

[12]. Subsequently, we replaced the selenoprotein population in this knockout mouse with either one of two mutant transgenes that produce tRNA gene products lacking i^6A and Um34, or mcm^5U and Um34 respectively, demonstrating that, although most of the selenoproteins were absent or diminished in the knockout mice, some were selectively replaced in the transgenic mice [8]. These replaced selenoproteins were housekeeping selenoproteins which are essential for liver function [8]. To assess the consequences of selenoprotein loss in $\Delta Trsp$ mice and their subsequent partial replacement with mutant transgenes, we examined gene expression in *Trsp*, $\Delta Trsp$, A34, G37L and G37H transgenic mice by microarray analysis. These analyses showed an elevated expression of several members of the phase II enzyme family in $\Delta Trsp$ mice. This change was validated through Q-PCR and Western blotting of the corresponding proteins. Several major phase II response genes that were up-regulated in $\Delta Trsp$ mice included *Gsta1*, *Gsta2*, *Gsta4*, *Gstm1*, *Gstm2*, *Gstm3*, *Cyp2a5*, *Ephx1*, *Hmox1* and *Aox1*.

Phase II enzymes conjugate xenobiotics or Phase I products to small donor molecules, such as glutathione, making them water soluble and easily excretable from the body, thus assisting in chemoprotection and detoxification [17]. They can be induced in animals by (i) chemical compounds which can react with a sulfhydryl group; (ii) regulation of common promoter elements [e.g. ARE (antioxidant responsive element)]; and (iii) reactions

leading to catalysis of electrophiles and ROS (reactive oxygen species) (reviewed in [17]). Induction of phase II enzymes in tissues has been shown to protect against carcinogens [18]. GST isoenzymes conjugate electrophilic compounds to glutathione, thus preventing their interaction with DNA [19], whereas EPHX1 is a bifunctional protein, that metabolizes polycyclic aromatic hydrocarbons [20] and mediates sodium-dependent uptake of bile acids [21]. HMOX1 is a cytoprotective enzyme, which degrades haem to biliverdin, which is further reduced to bilirubin [22], with both biliverdin and bilirubin acting as antioxidants [23]. CYP2A5 metabolizes toxic xenobiotic compounds, such as nitrosamines and aflatoxins [24,25], takes part in the degradation of bilirubin [26] and is induced during hepatic pathogenesis [27]. AOX1 is a molybdenum-containing flavoprotein which plays an important role in ethanol-induced hepatic lipoperoxidation [28]. The expression of AOX1 may determine the susceptibility of liver cells to some pharmacological agents and the levels of ROS produced under certain pathophysiological conditions [29]. The effect of dietary selenium on hepatic chemoprotective enzymes or xenobiotic enzymes in rodents have been extensively studied over the last few decades and results indicated a role of this element in the regulation of several phase II enzymes, including GST [30,31], EPHX [32] and HMOX1 [33]. Deficiency of selenium has been associated with an increase in these enzymes in rodents [30–33]. Our results indicate a similar elevation in the

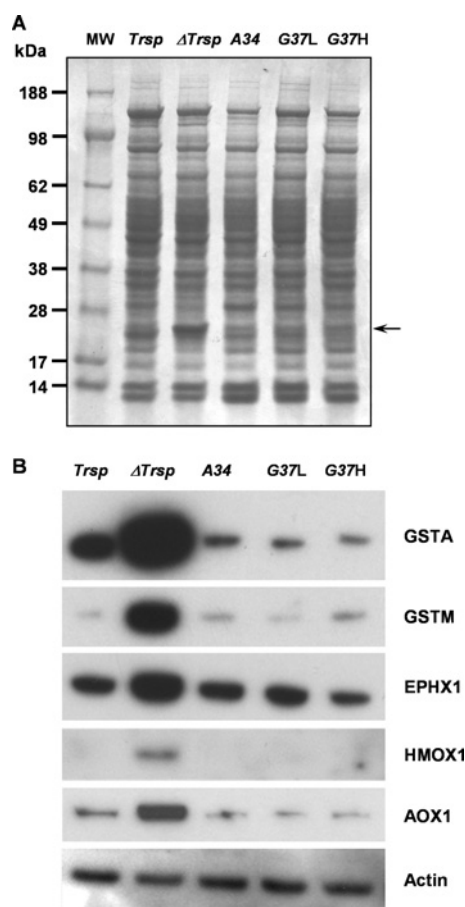


Figure 3 Western blot analysis of phase II enzymes

Protein extracts were prepared from liver of *Trsp*, Δ *Trsp*, A34, G37L and G37H mice and electrophoresed on 10% polyacrylamide gels. (A) Coomassie-Blue-stained gel. The elevated band corresponding to GST in Δ *Trsp* mice is indicated by an arrow on the right-hand side of the gel and molecular mass (MW) markers were run in lane 1 and their sizes indicated on the left-hand side. (B) The proteins were transferred on to PVDF membranes and the membranes were probed with antibodies specific for the indicated phase II enzymes and β -actin as described in the Experimental section. β -Actin served as a loading control.

levels of phase II enzymes in *Trsp* knockout mice, suggesting this phenomenon to be a result of loss of selenoproteins rather than a reduction in dietary selenium. Interestingly, the levels of phase II response genes were normal when housekeeping selenoproteins were replaced in transgenic mice, providing strong evidence of their up-regulation being a consequence of deficiency in housekeeping selenoproteins. In contrast, reduced expression of stress-related selenoproteins, such as GPx1 or SELR, had no role in the up-regulation of the phase II enzymes. An earlier study reported that inhibition of TR by aurothioglucose leads to induction of hepatic HMOX1 activity [34]. These investigators postulated that the lack of TR, or a TR-related reaction, induces hepatic HMOX1. GPx and GST are both responsible for detoxification of xenobiotic electrophiles by the addition of reduced glutathione (GSH) and possess similar enzyme folds in the GSH-binding site. Earlier studies have demonstrated that the Alpha-class GST isoenzymes also exhibit selenium-independent GPx activity in rodents [35,36], and these isoenzymes are very effective at reducing hydroperoxides, thus providing protection against membrane lipid peroxidation [37]. In the liver of Δ *Trsp* mice, the elevated levels of GST might functionally compensate for GPx and/or another selenoprotein(s) that might also be involved in detoxification.

The present study shows that an interplay exists between the loss of one or more housekeeping selenoproteins and enrichment in members of the phase II response protein class. The fact that several members of the phase II protein class manifesting a wide variety of functions are up-regulated suggests that several members of the housekeeping selenoprotein class are likely to be involved in this interplay. Thus our results provide strong evidence of a functional link between housekeeping selenoproteins and phase II enzymes.

This work was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health (D. L. H.), and by National Institutes of Health grants (V. N. G.).

REFERENCES

- Hatfield, D. L., Berry, M. J. and Gladyshev, V. N. (2006) Selenium: Its Molecular Biology and Role in Human Health, Springer Science Business Media LLC, New York
- Hatfield, D. L. and Gladyshev, V. N. (2002) How selenium has altered our understanding of the genetic code. *Mol. Cell Biol.* **22**, 3565–3576
- Kryukov, G. V., Castellano, S., Novoselov, S. V., Lobanov, A. V., Zehab, O., Guigo, R. and Gladyshev, V. N. (2003) Characterization of mammalian selenoproteomes. *Science* **300**, 1439–1443
- Berry, M. J. (2005) Insights into the hierarchy of selenium incorporation. *Nat. Genet.* **37**, 1162–1163
- Driscoll, D. M. and Copeland, P. R. (2003) Mechanism and regulation of selenoprotein synthesis. *Annu. Rev. Nutr.* **23**, 17–40
- Kim, L. K., Matsufuji, T., Matsufuji, S., Carlson, B. A., Kim, S. S., Hatfield, D. L. and Lee, B. J. (2000) Methylation of the ribosyl moiety at position 34 of selenocysteine tRNA[Ser]Sec is governed by both primary and tertiary structure. *RNA* **6**, 1306–1315
- Carlson, B. A., Xu, X. M., Gladyshev, V. N. and Hatfield, D. L. (2005) Selective rescue of selenoprotein expression in mice lacking a highly specialized methyl group in selenocysteine tRNA. *J. Biol. Chem.* **280**, 5542–5548
- Carlson, B. A., Moustafa, M. E., Sengupta, A., Schweizer, U., Shrimali, R., Rao, M., Zhong, N., Wang, S., Feigenbaum, L., Lee, B. J. et al. (2007) Selective restoration of the selenoprotein population in a mouse hepatocyte selenoproteinless background with different mutant selenocysteine tRNAs lacking Um34. *J. Biol. Chem.* **282**, 32591–32602
- Bosl, M. R., Takaku, K., Oshima, M., Nishimura, S. and Taketo, M. M. (1997) Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (*Trsp*). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5531–5534
- Kumaraswamy, E., Carlson, B. A., Morgan, F., Miyoshi, K., Robinson, G. W., Su, D., Wang, S., Southon, E., Tessarollo, L., Lee, B. J. et al. (2003) Selective removal of the selenocysteine tRNA [Ser]Sec gene (*Trsp*) in mouse mammary epithelium. *Mol. Cell Biol.* **23**, 1477–1488
- Hatfield, D. L., Carlson, B. A., Xu, X. M., Mix, H. and Gladyshev, V. N. (2006) Selenocysteine incorporation machinery and the role of selenoproteins in development and health. *Prog. Nucleic Acid Res. Mol. Biol.* **81**, 97–142
- Carlson, B. A., Novoselov, S. V., Kumaraswamy, E., Lee, B. J., Anver, M. R., Gladyshev, V. N. and Hatfield, D. L. (2004) Specific excision of the selenocysteine tRNA[Ser]Sec (*Trsp*) gene in mouse liver demonstrates an essential role of selenoproteins in liver function. *J. Biol. Chem.* **279**, 8011–8017
- Moustafa, M. E., Carlson, B. A., El-Saadani, M. A., Kryukov, G. V., Sun, Q. A., Harney, J. W., Hill, K. E., Combs, G. F., Feigenbaum, L., Mansur, D. B. et al. (2001) Selective inhibition of selenocysteine tRNA maturation and selenoprotein synthesis in transgenic mice expressing isopentenyladenosine-deficient selenocysteine tRNA. *Mol. Cell Biol.* **21**, 3840–3852
- Driscoll, D. M. and Chavatte, L. (2004) Finding needles in a haystack. *In silico* identification of eukaryotic selenoprotein genes. *EMBO Rep.* **5**, 140–141
- Gromer, S., Eubel, J. K., Lee, B. L. and Jacob, J. (2005) Human selenoproteins at a glance. *Cell Mol. Life Sci.* **62**, 2414–2437
- Rederstorff, M., Krol, A. and Lescure, A. (2006) Understanding the importance of selenium and selenoproteins in muscle function. *Cell Mol. Life Sci.* **63**, 52–59
- Talalay, P. (2000) Chemoprotection against cancer by induction of phase 2 enzymes. *Biofactors* **12**, 5–11
- Kwak, M. K., Egner, P. A., Dolan, P. M., Ramos-Gomez, M., Groopman, J. D., Itoh, K., Yamamoto, M. and Kensler, T. W. (2001) Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. *Mutat. Res.* **480–481**, 305–315

- 19 Hayes, J. D. and Pulford, D. J. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* **30**, 445–600
- 20 Fretland, A. J. and Omiecinski, C. J. (2000) Epoxide hydrolases: biochemistry and molecular biology. *Chem. Biol. Interact.* **129**, 41–59
- 21 von, D. P., Amoui, M., Alves, C. and Levy, D. (1993) Na⁺-dependent bile acid transport by hepatocytes is mediated by a protein similar to microsomal epoxide hydrolase. *Am. J. Physiol.* **264**, G528–G534
- 22 Otterbein, L. E. and Choi, A. M. (2000) Heme oxygenase: colors of defense against cellular stress. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **279**, L1029–L1037
- 23 Wu, T. W., Carey, D., Wu, J. and Sugiyama, H. (1991) The cytoprotective effects of bilirubin and biliverdin on rat hepatocytes and human erythrocytes and the impact of albumin. *Biochem. Cell Biol.* **69**, 828–834
- 24 Camus, A. M., Geneste, O., Honkakoski, P., Berezat, J. C., Henderson, C. J., Wolf, C. R., Bartsch, H. and Lang, M. A. (1993) High variability of nitrosamine metabolism among individuals: role of cytochromes P450 2A6 and 2E1 in the dealkylation of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in mice and humans. *Mol. Carcinog.* **7**, 268–275
- 25 Pelkonen, P., Lang, M. A., Negishi, M., Wild, C. P. and Juvonen, R. O. (1997) Interaction of aflatoxin B1 with cytochrome P450 2A5 and its mutants: correlation with metabolic activation and toxicity. *Chem. Res. Toxicol.* **10**, 85–90
- 26 Abu-Bakar, A., Moore, M. R. and Lang, M. A. (2005) Evidence for induced microsomal bilirubin degradation by cytochrome P450 2A5. *Biochem. Pharmacol.* **70**, 1527–1535
- 27 Camus-Randon, A. M., Raffalli, F., Berezat, J. C., McGregor, D., Konstandi, M. and Lang, M. A. (1996) Liver injury and expression of cytochromes P450: evidence that regulation of CYP2A5 is different from that of other major xenobiotic metabolizing CYP enzymes. *Toxicol. Appl. Pharmacol.* **138**, 140–148
- 28 Shaw, S. and Jayatilake, E. (1990) The role of aldehyde oxidase in ethanol-induced hepatic lipid peroxidation in the rat. *Biochem. J.* **268**, 579–583
- 29 Neumeier, M., Weigert, J., Schaffler, A., Weiss, T. S., Schmidl, C., Buttner, R., Bollheimer, C., Aslanidis, C., Scholmerich, J. and Buechler, C. (2006) Aldehyde oxidase 1 is highly abundant in hepatic steatosis and is downregulated by adiponectin and fenofibric acid in hepatocytes *in vitro*. *Biochem. Biophys. Res. Commun.* **350**, 731–735
- 30 Christensen, M. J., Nelson, B. L. and Wray, C. D. (1994) Regulation of glutathione S-transferase gene expression and activity by dietary selenium. *Biochem. Biophys. Res. Commun.* **202**, 271–277
- 31 Liu, J. Z., Zhang, B. Z. and Milner, J. A. (1994) Dietary selenite modifies glutathione metabolism and 7,12-dimethylbenz(a)anthracene conjugation in rats. *J. Nutr.* **124**, 172–180
- 32 Reddy, C. C., Thomas, C. E., Scholz, R. W. and Massaro, E. J. (1982) Effects of inadequate vitamin E and/or selenium nutrition on enzymes associated with xenobiotic metabolism. *Biochem. Biophys. Res. Commun.* **107**, 75–81
- 33 Mostert, V., Hill, K. E., Ferris, C. D. and Burk, R. F. (2003) Selective induction of liver parenchymal cell heme oxygenase-1 in selenium-deficient rats. *Biol. Chem.* **384**, 681–687
- 34 Mostert, V., Hill, K. E. and Burk, R. F. (2003) Loss of activity of the selenoenzyme thioredoxin reductase causes induction of hepatic heme oxygenase-1. *FEBS Lett.* **541**, 85–88
- 35 Hurst, R., Bao, Y., Jemth, P., Mannervik, B. and Williamson, G. (1998) Phopholipid hydroperoxide glutathione peroxidase activity of human glutathione transferases. *Biochem. J.* **332**, 97–100
- 36 Yang, Y., Sharma, R., Zimniak, P. and Awasthi, Y. C. (2002) Role of alpha class glutathione S-transferase as antioxidant enzymes in rodent tissues. *Toxicol. Appl. Pharmacol.* **182**, 105–115
- 37 Prabhu, K. S., Reddy, P. V., Gumprich, E., Hildenbrandt, G. R., Scholtz, R. W., Sordillo, L. M. and Reddy, C. C. (2001) Microsomal glutathione S-transferase A1-1 with glutathione peroxidase activity from sheep liver: molecular cloning, expression and characterization. *Biochem. J.* **360**, 345–354

Received 30 January 2008/13 March 2008; accepted 28 March 2008

Published as BJ Immediate Publication 28 March 2008, doi:10.1042/BJ20080277